intranasal doses of rapamycin. Percentage mean weight data of mice exposed to SEB, SEB+rapamycin (0.16 mg/kg intranasal) using 2 different treatment schedules after SEB exposure are shown. Rapamycin (0.16 mg/kg) was administered intranasally to mice at (ii) 5, 24, 48, and 72 h; (iii) 17, 23, 41, 65, and 89 h. Points represent the % mean weight change for each group (n=10).

[0035] FIG. **14** demonstrates survival of mice treated with (i) SEB, (ii) SEB+rapamycin (0.16 mg/kg intranasal) at (i) 5, 24, 48, 72, and 96 h (Ri); (ii) 5, 24, 48, and 72 h (Rii); (iii) 17, 23, 41, 65, and 89 h (Riii). Time to death is in h after SEB exposure.

[0036] FIG. **15** demonstrates rapamycin prevents apoptosis of cells from C3H/HeJ mice treated with SEB. Fluorescent counts of cells from peripheral blood or spleen of mouse treated with DMSO (negative drug control), SEB and SEB+ rapamycin (0.16 mg/kg intranasal) at 17 h after SEB exposure are shown. Cells were isolated from mice at 18 h after SEB exposure and stained with JC-1 dye. Flow cytometer was used to measure fluorescence and fluorescence data at 530 nm representing cellular apoptosis from peripheral blood or spleen taken from rapamycin treated and untreated mice are shown.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0037] The present invention is rooted in the inventors' discovery of rapamycin's therapeutic effects in preventing and treating toxic shocks, especially toxic shocks induced by *Staphylococcal* exotoxins, such as *Staphylococcal* enterotoxin B-induced toxic shocks. The inventors demonstrate, for the first time, that rapamycin effectively inhibited SEB-mediated production of TNFα, IL-1β, IL-6, IL-2, IFNγ, MCP-1, MIP-1α, and MIP-1β by human PBMC in vitro. Besides decreasing the levels of proinflammatory cytokines in vitro, SEB-induced proliferation of T cells was also completely blocked by rapamycin. As excessive release of cytokines mediates the pathogenic effects of SEB in vivo, the use of rapamycin, an immunosuppressent was tested in vivo. For the first time, the present inventors demonstrate that rapamycin was 100% effective in protecting mice from SEB-mediated shock.

[0038] Previous studies of drug treatment for SEB-mediated shock models indicate a very narrow therapeutic window of treatment with different types of inhibitors to reduce the biological effects of SEB (1, 29, 30, 33, 58). For example, using the potent steroid dexamethasone, survival of mice in this model of SEB-induced toxic shock was 100%, 70%, 30%, 10% when mice were treated with dexamethasone at 2, 3, 4, 5 h, respectively, after SEB treatment (Table 1). As demonstrated in this application, rapamycin, provides even a wider therapeutic window, such that when given at up to 24 hours following SEB exposure, still afforded 100% protection against mortality, temperature, and weight fluctuations. Serum levels of MCP-1 and IL-6 were also markedly reduced when mice were treated with rapamycin compared to control SEB-exposed mice. These results reveal the potency of rapamycin even when given post SEB exposure. The serum cytokines, temperature, and weight data revealed the protective effects of rapamycin after SEB exposure. The high levels of IL-2 in vivo with rapamycin treatment appear to be transient and did not affect survival.

[0039] The rapamycin doses used in vivo in the present application are in the same range as those used in the field to reduce murine adjuvant arthritis and carcinogen-induced lung tumors (7, 19). Peak blood concentrations of rapamycin also agree with those used in murine models (19). In healthy subjects, blood concentrations of rapamycin reached to 78.2±18 ng/ml 1 h after 15 mg of rapamycin (47). The rapamycin dose used clinically to prevent graft rejection in renal transplantation is much lower (5 mg/day) with blood levels of 37.4±21 ng/ml at 1.8 h. However rapamycin is used for extended periods of years in these patients where long term accumulative toxic effects have to be taken into consideration. The doses in the present application are similar to those used in animal models of disease and the short course of treatment in this study suggests that this drug can be transition to human trials to treat SEB-induced shock. As such, a therapeutically effective amount of rapamycin via different administration routes for treating toxic shocks is within the purview of one of ordinary skill in the art. For example, an intranasal dose ranging from 0.08 mg/kg-0.7 mg/kg is effective; and an intraperitoneal dose ranging from 0.3 to 1.6 mg/kg is effective.

[0040] Because rapamycin has been approved by FDA and safely administered to critically ill transplant patients without significant toxicity even after 2 years of use, the application of rapamycin against toxic shock in humans is promising.

[0041] The following examples are given to illustrate the present invention. It should be understood, however, that the spirit and scope of the invention is not to be limited to the specific conditions or details described in these examples. All references identified herein are hereby expressly incorporated by reference.

EXAMPLES

Example 1

[0042] Materials and Methods

[0043] Reagents. Purified SEB was obtained from Toxin Technology (Sarasota, Fla.). The endotoxin content of these preparations was <1 ng of endotoxin/mg protein, as determined by the Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, Md.). Human cytokine ELISA kits and assay reagents were purchased from R&D Systems (Minneapolis, Minn.). Mouse cytokine enzyme-linked immunosorbent assay (ELISA) reagents were obtained from Pharmingen (San Diego, Calif.). Rapamycin and all other reagents were from Sigma (St. Louis, Mo.). Rapamycin was prepared at 35 mg/ml in DMSO and diluted in saline prior to use.

[0044] Cell cultures. Human PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of heparinized blood from normal human donors. The PBMC (10^6 cells/ml) were cultured at 37° C. in 24-well plates containing RPMI 1640 medium and 10% heat-inactivated fetal bovine serum. Cells were stimulated with SEB (200 ng/ml) for 16 h and the supernatants were harvested and analyzed for TNFα, IL-1β, IL-6, IL-2, IFNγ, MCP-1, MIP-1α and MIP-1β by ELISA as described previously (26, 27). Rapamycin, when present, was added simultaneously with SEB.

[0045] Human T-cell proliferation assays. PBMC (10^5 cells/well) were plated in triplicate with SEB (200 ng/ml), with or without varying concentrations of rapamycin, for 48 h at 37° C. in 96-well microtiter plates. Cells were pulsed with 1 μCi/well [^{3}H]thymidine (New England Nuclear, Boston, Mass.) during the last 5 h of culture, as previously described (26). Cells were harvested onto glass fiber filters, and incorporated [^{3}H]thymidine was measured by liquid scintillation.